TECHNICAL DATA SHEET

ROSE BENGAL CHLORAMPHENICOL AGAR

DETECTION AND ENUMERATION OF YEASTS & MOLDS

1 INTENDED USE

Rose Bengal Chloramphenicol Agar is recommended for the selective isolation and enumeration of yeast and molds in food products, environmental, and any other potentially contaminated samples of animal origin.

2 HISTORY

In 1962, work done by Mossel on the detection of yeast and molds in foods demonstrated the superiority of media at a neutral pH containing an antibacterial agent over those based on an acid pH. In 1973, Jarvis developed and used with success Rose Bengal Chlortetracycline. During the course of subsequent work in 1978 by Korburger and Rogers, and in 1981 by Baggerman, chloramphenicol or gentamicin replaced chlortetracycline. Current formulations contain chloramphenicol.

3 PRINCIPLES

Papaic digest of soybean meal and glucose assure the growth of yeast and molds.

Rose Bengal inhibits the development of bacteria and prevents the invasion of molds on the Petri dish, by limiting their proliferation. Assimilated by yeasts, it facilitates their enumeration by coloring the colonies pink.

The presence of chloramphenicol, a thermostable antibiotic, allows a reinforcement of the selectivity against the majority of contaminating bacteria.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance. .

For 1 liter of media :

- Papaic digest of soybean meal ..... 5,0 g
- Glucose ..... 10,0 g
- Monopotassium phosphate ..... 1,0 g
- Magnesium sulfate, 7 H_2O ..... 0,5 g
- Rose Bengal ..... 50,0 mg
- Chloramphenicol ..... 0,1 g
- Bacteriological agar ..... 13,0 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

5 PREPARATION

- Dissolve 29,7 g of dehydrated media (BK151) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense into vials at 100 mL per vial.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.

✓ **Reconstitution : 29,7 g/L**

✓ **Sterilization : 15 min at 121 °C**

6 INSTRUCTIONS FOR USE

- Dry the plates in an incubator with the covers partially removed.
- To the surface of the media prepared as above, transfer 0,1 mL of the sample to test and its serial dilutions.
- Spread the inoculum on the surface with the aid of a sterile triange or « hockey stick ».
- Incubate at 20-25 °C for 3 to 5 days, shielded from light.

✓ **Inoculation :**
0,1 mL on surface

✓ **Incubation :**
3 to 5 days at 20-25 °C

7 RESULTS

Enumerate the yeasts and molds..
Yeast colonies appear pink due to the assimilation of the Rose Bengal dye.
Yeasts and non-inhibited bacteria can be mistaken for one another. It is necessary to confirm identity under the microscope.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : cream to pinkish powder, fluid and homogeneous.
Prepared media : pink agar.

Typical culture response after 5 days of incubation at 25 °C.

Microorganisms		Growth (Productivity Ratio : P_R)
Saccharomyces cerevisiae	WDCM 00058	$P_R \geq 50$ %
Candida albicans	WDCM 00054	$P_R \geq 50$ %
Aspergillus brasiliensis	WDCM 00053	$P_R \geq 50$ %
Escherichia coli	WDCM 00013	Inhibited, score 0
Bacillus subtilis	WDCM 00003	Inhibited, score 0
Staphylococcus aureus	WDCM 00034	Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in vials (*) : 90 days at 2-8 °C.
Prepared media in plates (*) : 7 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK151HA

11 BIBLIOGRAPHY

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Korburger, J.A. and Rodgers, M.F.. 1978. Single or multiple antibiotic-amended media to enumerate yeasts and moulds. Journal of Food Protection, **41** : 367-369.

Baggerman, W.I.. 1981. A modified Rose Bengal medium for the enumeration of yeasts and moulds from foods. European Journal of Applied Microbiology and Biotechnology, **12** : 242-247.

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Mislivec, P.B., Beuchat, L.R. and Cousin, M.A.. 1992. Yeasts and Molds. *In* Compendium of methods for the microbiological examination of foods, 3rd Ed. American Public Healh Assoc., Washington D.C : 239-249.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : ROSE BENGAL CHLORAMPHENICOL AGAR_ENv6
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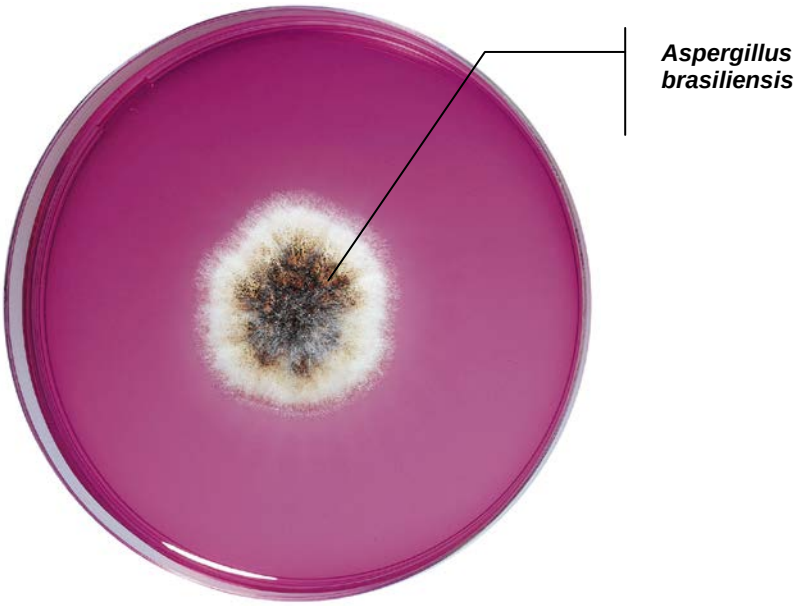
ANNEX 1 : PHOTO SUPPORT

Rose Bengal Chloramphenicol Agar

Detection and enumeration of yeasts and molds.

Results :

Growth obtained after 3 to 5 days of incubation at 20-25 °C.



Characteristics :
Good growth with sporulation (molds).
The yeasts appear pink (assimilation of the Rose Bengal).